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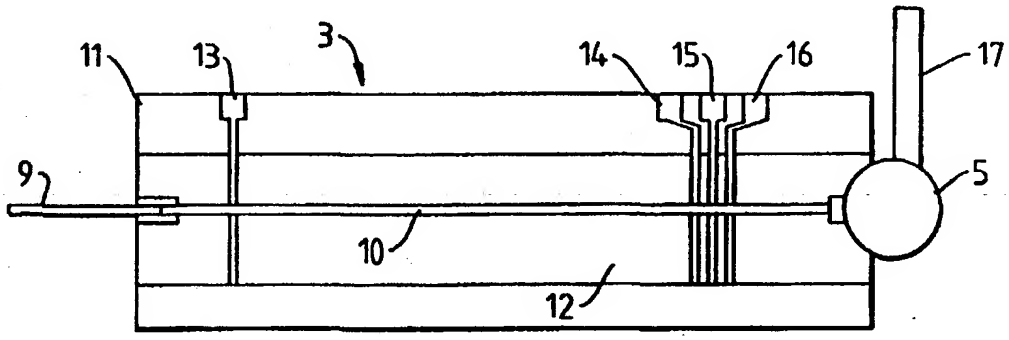

(57) Abstract

A metal micro-electrode array is deposited by means such as photolithography on a non-conducting substrate and placed in contact with a flowing fluid stream. A staircase voltage waveform, optionally with cleaning or conditioning pulses, is applied to the electrode and the current response of the fluid is measured to enable analysis of the stream.

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ELECTRO-CHEMICAL DETECTOR

The present invention relates to electro-chemical detectors and especially to the use of such detectors for analysing a flowing stream of a fluid.

There are several known techniques used in chemical analysis, such as capillary zone electrophoresis and liquid chromatography, which produce, as an output, a liquid stream whose constitution varies with time. Such a stream may, in some cases, be regarded as a succession of discrete samples, each sample containing either one or a mixture of components. It is desirable to be able to perform measurements on each sample as it passes a detector, but prior art devices have not always been sufficiently sensitive to distinguish between samples nor sufficiently responsive to perform a number of measurements on a single sample as it passes the detector. There is therefore a need for a more sensitive and responsive detector.

According to the present invention there is provided: a method of performing measurements on a flowing fluid, the method comprising the steps of:

providing a metal micro-electrode in or adjacent to the path of said fluid;

applying a time-varying signal to said micro-electrode; and

measuring the response of said fluid to said varying signal.

The present invention also provides: an apparatus for performing measurements on a flowing fluid, the apparatus comprising:

a metal micro-electrode array;

means for conducting said flowing fluid to flow adjacent to, or in contact with, said micro-electrode array;

means for applying a varying signal to said micro-electrode array; and

means for measuring the response of said fluid to the applied signal.

The use of a metal micro-electrode, which is preferably made of a noble metal such as gold or platinum, or other metal such as copper, allows different samples to be distinguished and also different measurements to be performed on each sample, thus vastly increasing the amount of information available from analysis of a fluid stream.

The electrode is preferably made of platinum, for example deposited on a silicon wafer by photolithography. The applied signal preferably varies in voltage and causes a changing current response which is measured. The signal is preferably varied with a period less than the time taken for each sample to cross the electrode region. In a preferred embodiment the signal is varied in a series of steps, or is a continuously varying waveform.

The present invention will be further described hereinafter with reference to the following description of exemplary embodiments and the accompanying drawings, in which:

Fig. 1 is a schematic view of a first embodiment of the invention;

Fig. 2 is an schematic view of electrodes suitable for use in the present invention;

Fig. 3 is an enlarged view of the electrode pattern, including the micro-electrode array, suitable for use in the present invention;

Fig. 4 shows a simple waveform applied to the micro-electrode array in a first embodiment of the invention;

Fig. 5 shows results achieved with the waveform of Fig. 4.

Fig. 6 shows a waveform with cleaning pulses applied to the micro-electrode in a second embodiment of the invention;

Fig. 7 shows results achieved with the waveform of Fig. 6;

Fig. 8 shows a waveform with cleaning pulses applied to the micro-electrode in a third embodiment of the invention;

Fig. 9 shows results achieved with the waveform of Fig. 8;

Fig. 10 shows a simple waveform applied repeatedly during HPLC;

Fig. 11 shows the results achieved with the waveform of Fig. 10; and

Fig. 12 shows the detector output (an electropherogram) obtained during the CZE separation of a mixture of catecholamines.

In the drawings, like parts are denoted by like reference numerals.

Figure 1 shows, schematically, an embodiment of the invention. A capillary zone electrophoresis (CZE) apparatus 1 outputs a liquid stream 2. This stream is generated by

separating out the constituents of a specimen under investigation and thus the composition of the stream varies along its length, or with time if a stationary point is observed. The stream might also be generated by any other suitable apparatus such as a liquid chromatography column or it might represent a sample drawn from a pipeline or a reaction vessel.

The sample stream 2 passes over an electrode array 3, which is described in more detail below. The effluent stream 4 is passed to a waste container or drain 5 though it may be returned to the pipeline or reaction vessel depending on the application.

A control unit 7, which may be a computer or dedicated hardware, provides an analysis signal to the electrode array and analyses the response. Power is provided by a power supply 6 and the results are passed to a display or storage device 8 such as a video monitor, printer, chart recorder, plotter or disk drive.

Figure 2 shows the electrode arrangement in greater detail. The liquid stream 2 arrives via a capillary tube 9 from the CZE apparatus and flows down a channel 10 defined by the electrode substrate 11 and a groove in a cap 12. The electrode substrate 11 is fabricated from a silicon wafer and cap 12 is made of Corning glass. They are joined using a photoresist. If an adhesive is used it is important to ensure that it does not flow into the capillary channel. An alternative, but expensive, procedure would be to use a solid low melting point glass target to sputter a layer of glass onto the cap and substrate, followed by an anodic bonding process.

The electrodes 13, 14, 15 and 16 comprise platinum deposited on a chromium adhesion layer and are printed onto the substrate, before the cap is added, by photolithography. Other noble or non-noble metals may be used. Electrode 13 is an earthing electrode provided to isolate the analytical electrodes from the high voltages used in CZE. It may be omitted. Electrode 15 is the micro-electrode array which will be described in more detail below. Electrodes 14 and 16 are guard or auxiliary electrodes. Pads are provided to enable electrical connections to the electrodes to be made.

The electrode may also be constructed by screen printing or by building a multilayered "sandwich" of alternating metal foil and insulators. If screen printing is used the metal ink contains only about 80% metal, the remainder being binding materials. With the sandwich method, the edge of it, which may be polished to provide a flat surface for the detector cap, comprises an array of micro-electrodes separated by insulators. Connections may be made to another edge of the sandwich. The width and separation of the micro-electrodes in the sandwich method may be increased by cutting the edge at an angle.

As shown in figure 3 the micro-electrode array consists of 8 micro-electrodes of 5 μm width at a spacing of 5 μm. The first guard electrode is spaced, D_1, 10 mm from the earthing electrode. This distance should be large enough to ensure isolation of the analytical electrodes but, for convenience, should not be too large. The guard electrodes 16 and 16 are spaced, D_2, 100 μm from the micro-electrode array and have a width, D_3, of 100 μm.

The precise number width and spacing of the micro-electrodes will vary between applications. The width may be in the range of from 0.1 to 50 μm and the spacing in the range of from 0.1 to 100 μm.

In use, a time-varying potential difference signal is applied between the micro-electrode array and a second electrode. This may be done either in a two electrode mode, in which the second electrode is a reference electrode, or a three electrode mode in which the second electrode is one or both of the auxiliary electrodes which are patterned on the device. The reference electrode may either be patterned on the device in a similar position or instead of the auxiliary electrodes or may be external to the device, as shown at 17 in Figure 2.

The current produced at the micro-electrode array by reaction of the analyte is measured and provides the necessary information for the analysis. The voltage signal is varied to enable the response of the fluid to be measured at different voltages to extract maximum information. The fluid stream provided by the CZE apparatus may be regarded as a succession of separate samples, each of which may contain one or more components which are to be analysed. It is thus important that the complete set of measurements be taken in a time appreciably less than the time taken for the sample to cross the analytical electrode array. Thus the rate of change of the analytical signal applied to the micro-electrode must be substantially greater than the rate of flow of the fluid stream. The scanning rate of the analytical signal is limited by the characteristics of the electrode array, particularly its

capacitance, which depends upon its dimensions, and the sensitivity of the current measuring equipment.

Figure 4 shows a simple staircase waveform which might be used with the apparatus. In this waveform the voltage is decreased from +0.3V to -1.4V in 100mV steps at a rate of 1 step per second. Figure 5 shows the current response from a 10 ml solution of HNO_3 (0.1 M) as samples of copper ions were added. The data points are as follows:

□ - blank Nitric Acid;

x - after the addition of 1 ml 0.1 M copper;

◇ - after the addition of 2 ml 0.1 M copper.

Figure 6 shows an alternative waveform which includes cleaning pulses between adjacent steps. The cleaning pulses comprise an oxidation pulse, to 0.6 V, followed by a reduction pulse, to -1.5 V. The oxidation pulse removes any metals deposited on the electrode while the reduction pulse reduces the surface oxidised platinum. The aim is to return the electrodes to their original state between steps. Figure 7 shows the test results, using the same symbols as figure 5. This waveform can give rise to an apparent increase in noise when metal ions are added. This is probably due to the negative potential pulse causing the metal ions to plate onto the electrode surface and also causing adsorption of hydrogen.

A further alternative waveform, which appears to give the best results, is shown in figure 8. In this waveform only the oxidation pulse, to 0.6V, is applied, the reduction pulse is omitted. The test results using this waveform are shown if figure 9, in which the data points are labelled as follows:

+ - blank Nitric Acid;

□ - after the addition of 1 ml 0.1 M copper;

◇ - after the addition of 1 ml 0.1 M copper and 1 ml 0.1 M cadmium;

Ɪ - after the addition of 1 ml 0.1 M copper, 1 ml 0.1 M cadmium and 1 ml 0.1 M lead.

As well as the cleaning pulses described above, similar pulses may be applied to precondition the electrode surface to favour the analysis of a particular substance, eg the hydroxy groups which form on a platinum electrode between 0.2 and 0.5V facilitate the oxidation of certain analytes such as carbohydrates and alcohols.

Whichever of the waveforms is used , it is applied to the sample repeatedly, usually with no pause between repetitions.

Figure 10 shows a simple voltage staircase waveform, form 500 - 1300 mV in 100mV steps of 100ms duration, which was applied repeatedly to the electrodes during the narrow bore HPLC of three catecholamines.

The three catecholamines (hydroquinone, dopamine and catechol, all 5mM) were separated on a Tachsphere 5ODS reverse phase column (15 cm x 3.9 mm, HPLC Technology Ltd) at ambient temperature using a flow rate of 0.66 ml min^{-1}. The eluant was a 70:30 mixture of pH3 phosphate/citrate buffer and methanol.

Fig. 11 shows the results obtained, clearly showing the separation of the three catecholamines.

In the above described embodiments, the voltage of the applied signal is varied to carry out the different measurements on the sample stream. However, depending on the characteristic of the sample it is desired to measure, any

other parameter of the signal, eg current, frequency or polarity, may be varied.

Fig. 12 shows, as an example, the output from the detector (an electropherogram) during a CZE separation of a mixture of catecholamines. The separation was carried out in a capillary with an internal diameter of 50 μm in an off-column detection mode (i.e. using an earthing electrode other than the one on the detector) with a distance of 3.8 cm between the earth and the detector electrodes and at a field strength of 350 V cm^{-1}. The separation buffer contained 2[N-morpholino]ethanesulphonic acid (concentration = 10 mM, pH = 7.0, adjusted by the addition of solid NaOH) and the detection potential was 0.8 V. The separation mixture of catecholamines contained dopamine (conc. = 0.6 mM, arteranol (0.8 mM), isoproteranol (0.7 mM) and hydroquinone (1.3 mM). The detection potential was 0.8 V vs Ag/AgCl.

CLAIMS

1. A method of performing measurements on a flowing fluid, the method comprising the steps of:

providing a metal micro-electrode in or adjacent to the path of said fluid;

applying a time-varying signal to said micro-electrode; and

measuring the response of said fluid to said varying signal.

2. A method according to claim 1 wherein the rate of variation of said signal is greater than the rate of variation of the fluid.

3. A method according to claim 1 or 2 wherein the applied signal varies periodically.

4. A method according to claim 1, 2 or 3 wherein the voltage of the applied signal is varied.

5. A method according to any one of the preceding claims wherein the response of the fluid is measured by measuring the current flowing through said electrode.

6. A method according to any one of the preceding claims wherein the period of the applied signal is less than the time taken for a part of the fluid to cross the electrode region.

7. A method according to any one of the preceding claims wherein said applied signal comprises a succession of periods, each of a predetermined duration, in which the variable parameter of the signal is held constant.

8. A method according to any one of the preceding claims wherein said applied signal includes cleaning or electrode conditioning pulses.

9. A method according to claim 8 wherein said cleaning or electrode conditioning pulses include at least a positive going voltage pulse.

10. A method according to claim 8 or 9 wherein said cleaning or electrode conditioning pulses include at least a negative going voltage pulse.

11. A method according to claim 8, 9 or when appendant on claim 7, wherein the duration of said cleaning or electrode conditioning pulses is less than said predetermined duration.

12. A method according to any one of the preceding claims wherein the flowing fluid is the output of a separating device such as a CZE or liquid chromatography device.

13. A method according to any one of the preceding claims wherein the metal micro-electrode is made of a noble metal, preferably gold or platinum.

14. An apparatus for performing measurements on a flowing fluid, the apparatus comprising:

a metal micro-electrode array;

means for conducting said flowing fluid to flow adjacent to, or in contact with, said micro-electrode array;

means for applying a varying signal to said micro-electrode array; and

means for measuring the response of said fluid to the applied signal.

15. An apparatus according to claim 14 wherein said metal is a noble metal, preferably platinum or gold.

16. An apparatus according to claim 14 or 15 wherein said means for applying comprises a voltage source for generating a signal of varying voltage.

17. An apparatus according to claim 14, 15 or 16 wherein said means for measuring comprises means for measuring the current produced at said micro-electrode array.

18. An apparatus according to claim 14, 15, 16 or 17 wherein the period of the applied signal is less than the time taken for a part of the fluid to cross the electrode region.

19. An apparatus according to any one of claims 14 to 18 further comprising a separating device, such as a CZE or liquid chromatography device, and wherein the flowing fluid is the output of said device.

20. An apparatus according to any one of claims 14 to 19 further comprising at least one auxiliary electrode adjacent said micro-electrode and wherein said applied signal is applied between said micro-electrode array and the or each auxiliary electrode or an external reference electrode.

21. An apparatus according to any one of claims 14 to 20 further comprising an earth electrode in electrical contact with said flowing fluid upstream of said micro-electrode array.

22. An apparatus according to any one of claims 14 to 21 further comprising a non-conductive substrate on which said electrodes array is formed, the substrate preferably comprising a ceramic or a silicon wafer.

23. An apparatus according to any one of claims 14 to 22 wherein the or each electrode is formed on said substrate by photolithography or screen printing.

Fig.1.

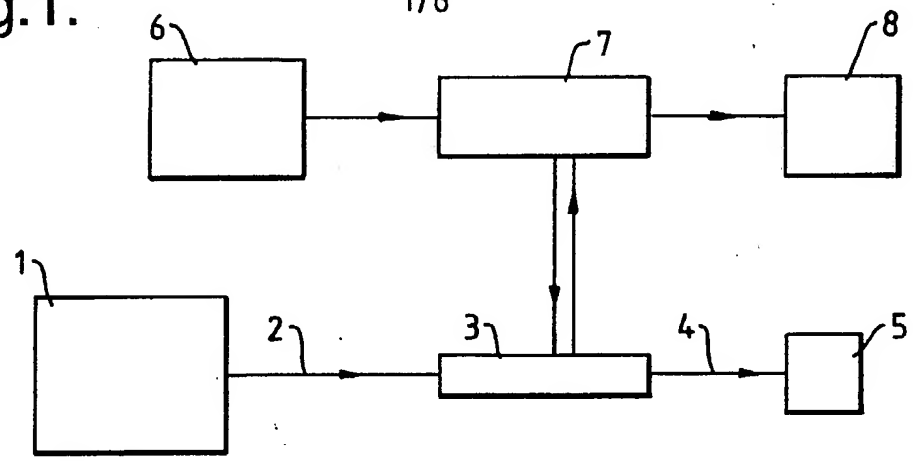

Fig.2.

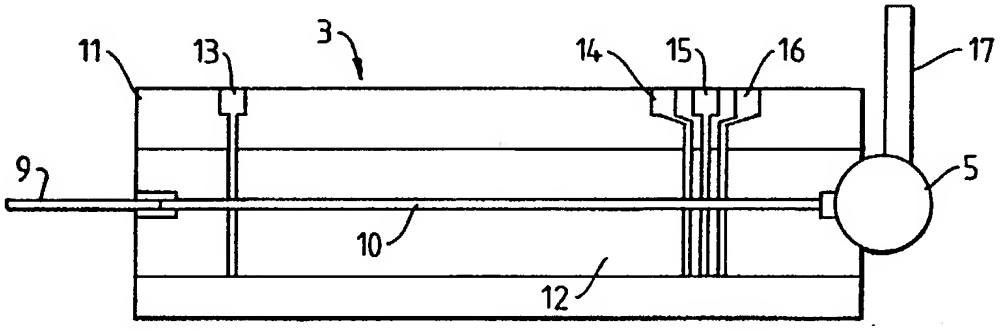

Fig.3.

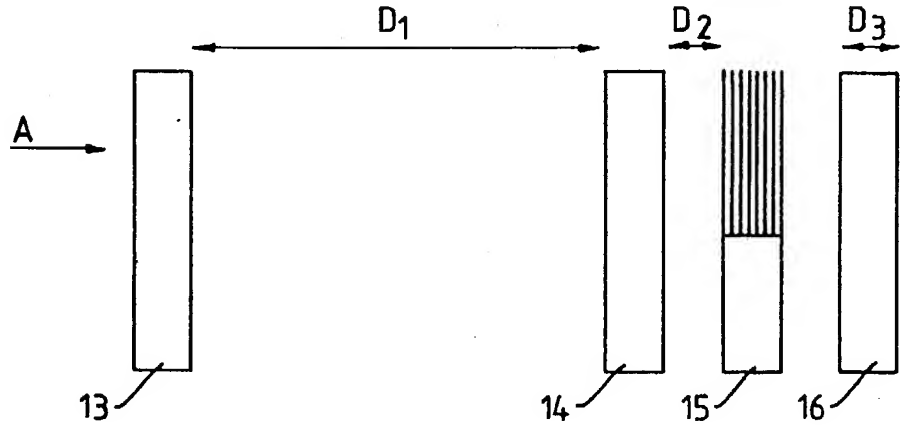

Fig.4.

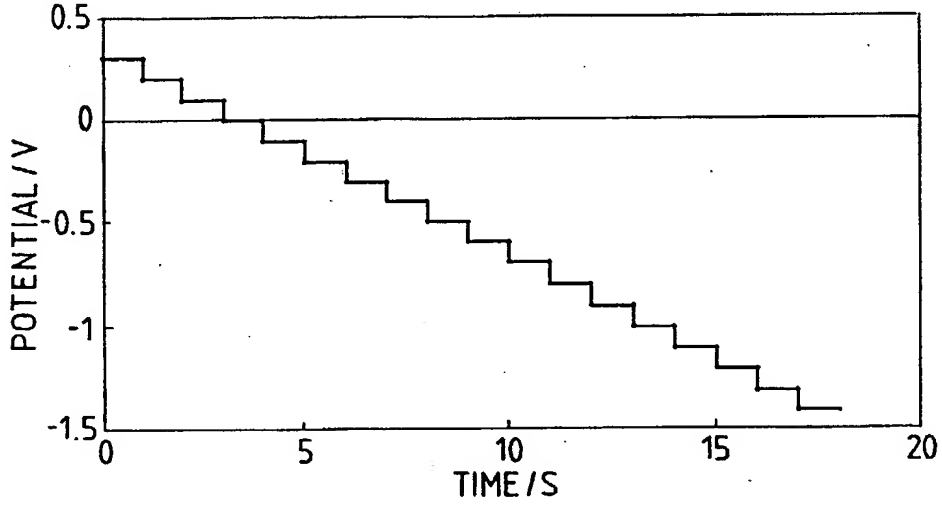
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Fig.5.

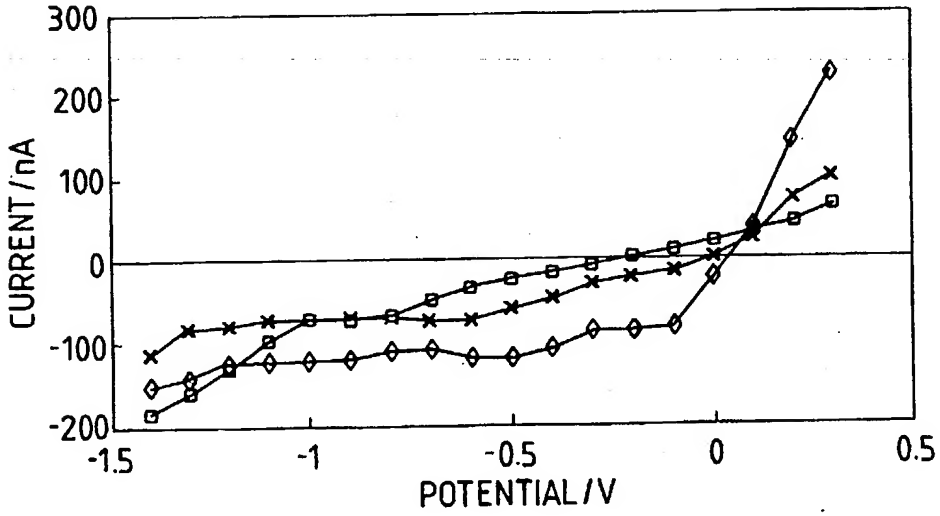
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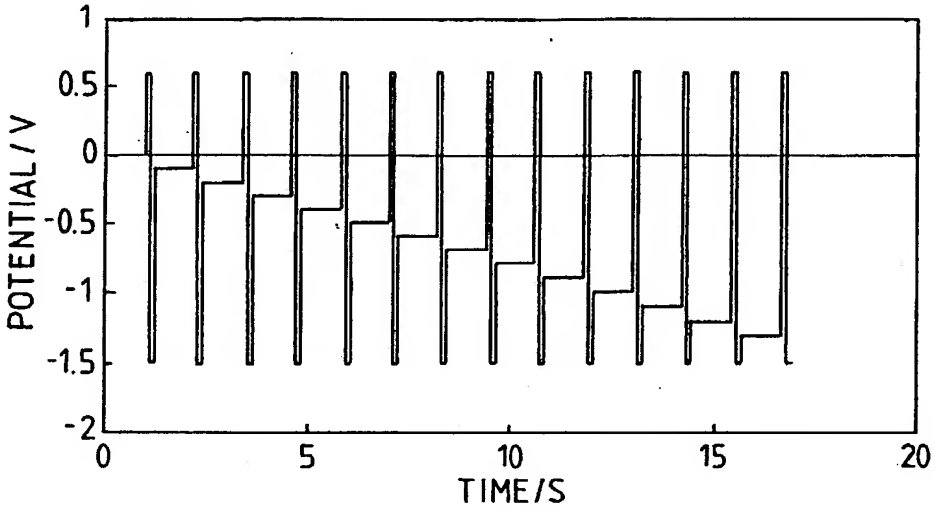
Fig.6.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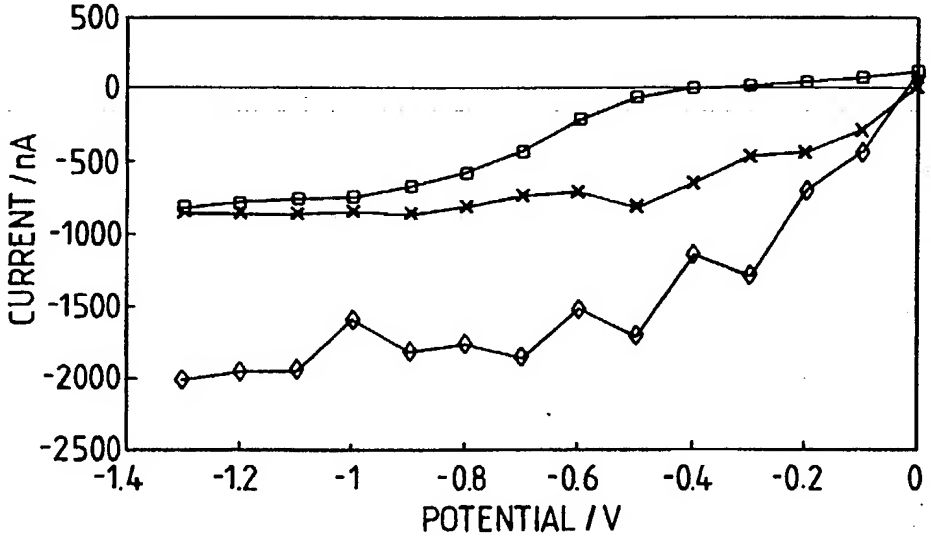
TIME/S

Fig.7.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POTENTIAL / V

Fig.8.

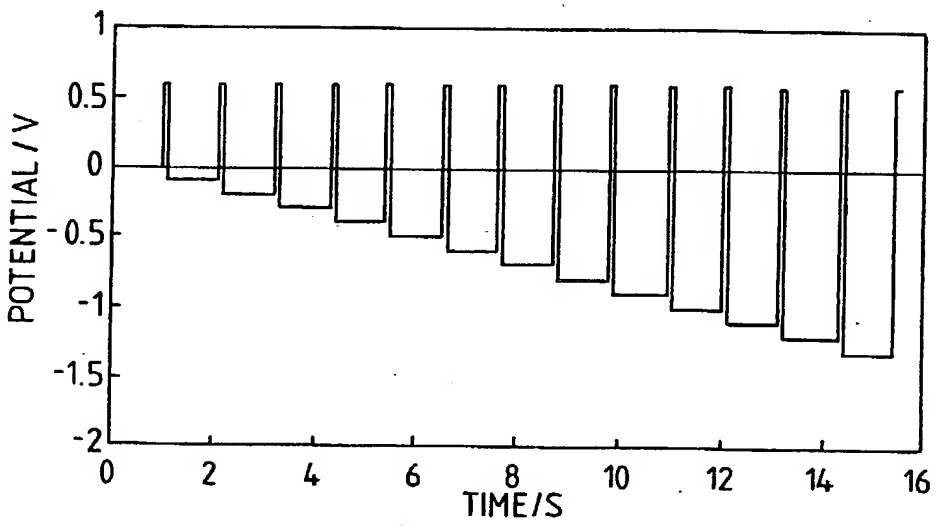
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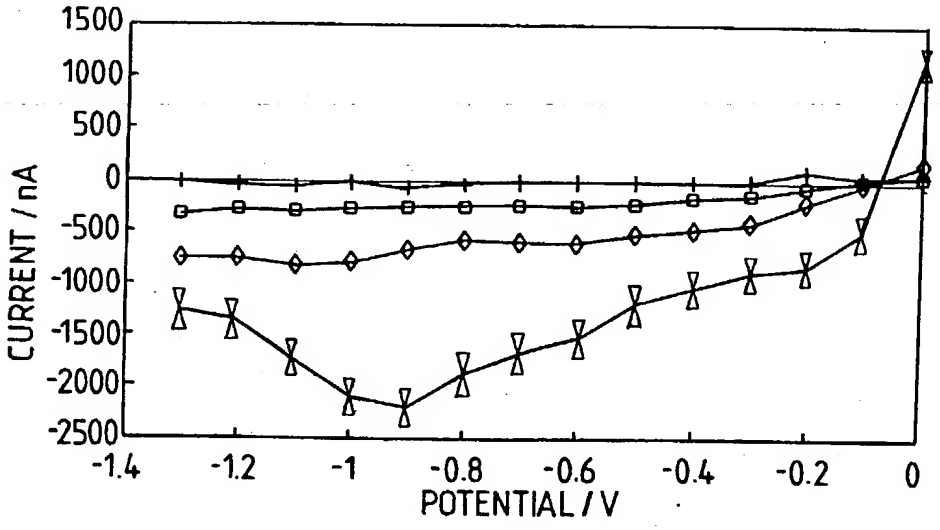
Fig.9.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POTENTIAL / V

Fig.10.

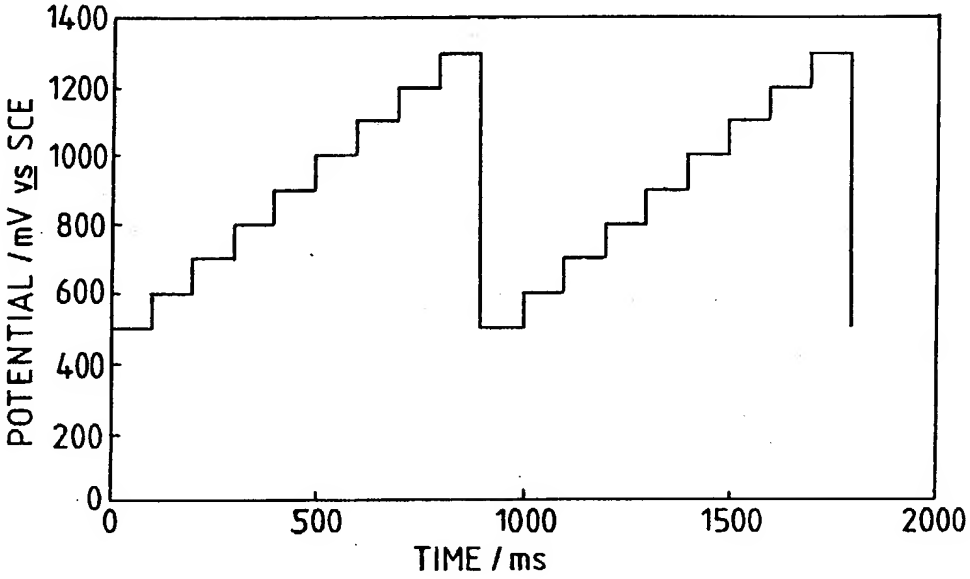
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Fig.12.

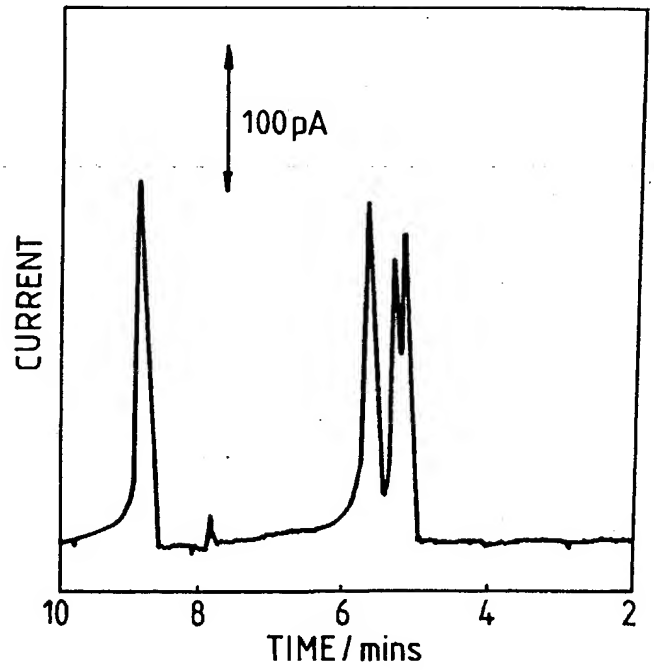
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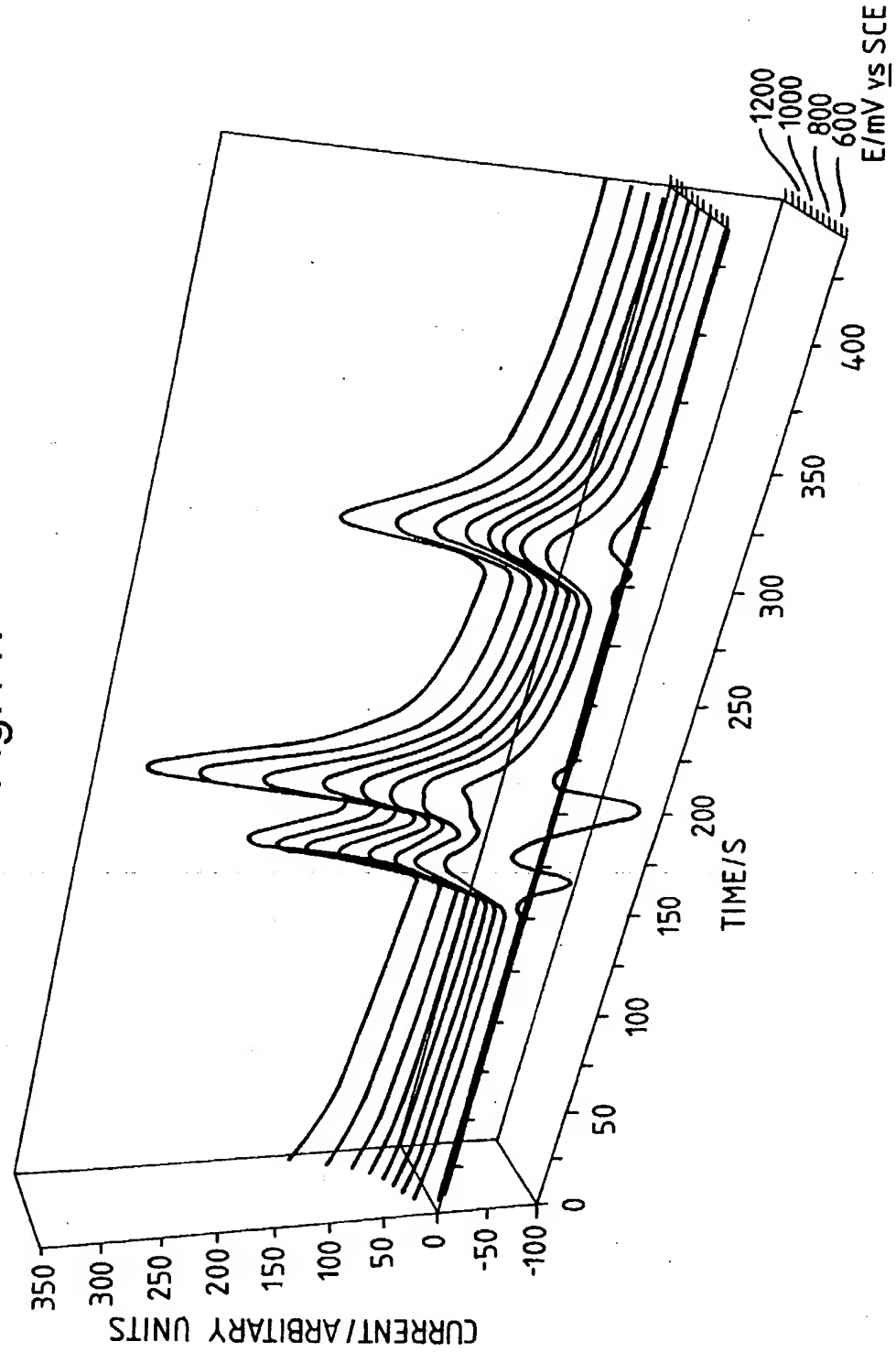
Fig.11.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INTERNATIONAL SEARCH REPORT

Inter... Application No
PCT/GB 94/02089

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF CHROMATOGRAPHY, vol.585, no.1, 25 October 1991, AMSTERDAM, NL pages 139 - 144, XP242212 Y.F. YIK 'MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY OF VITAMINE B6 WITH ELECTROCHEMICAL DETECTION' see figure 2 ---	1
Y	US,A,5 169 510 (S. M. LUNTE) 8 December 1992 see abstract; figure 1 ---	1
A	EP,A,0 475 713 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 18 March 1992 see abstract; figure 5 ---	1
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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 December 1994	10. 01. 95

Name and mailing address of the ISA	Authorized officer
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Inter. ..onal Application No
PCT/GB 94/02089

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CH,A,659 327 (INSTITUT ELEKTROKHIMII AKADMII NAUK SSSR) 15 January 1987 see abstract; figure 1	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intn. ional Application No
PCT/GB 94/02089

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5169510	08-12-92	NONE		
EP-A-0475713	18-03-92	US-A-	5126023	30-06-92
		CA-A-	2051006	11-03-92
		JP-A-	4244955	01-09-92
		US-A-	5298139	29-03-94
CH-A-659327	15-01-87	NONE		